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Biocontrol of Sclerotinia stem rot in soybeans with Sporidesmium sclerotivorum

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Abstract: *Sclerotinia stem rot of soybeans (also known as white mold) is caused by a soil-borne fungus and has become a serious problem in northern Iowa. Another fungus,* Sporidesmium sclerotivorum*, acts as a parasite of the sclerotia and this research tested whether this mycoparasite could act as an effective deterrent to the soybean stem rot pathogen.*

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Background

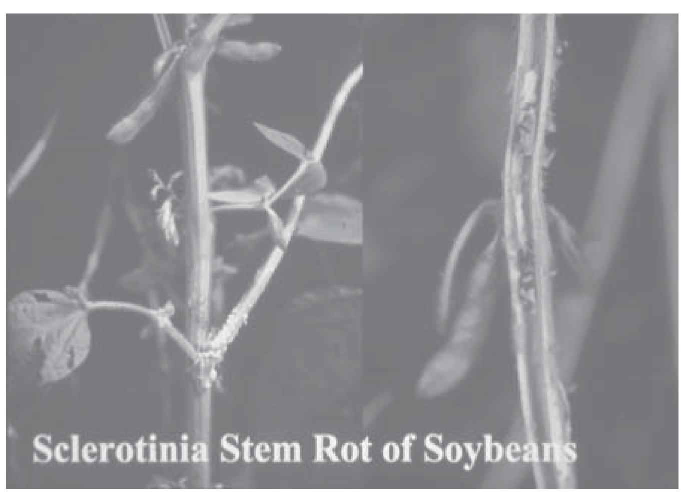
Sclerotinia stem rot of soybeans (also known as white mold) has been seen in Iowa for 50 years and is considered the second most important disease of soybeans in the north central region of the United States. It has become a serious problem for northern Iowa farmers in recent years. Areas of fields severely affected by white mold experienced documented yield losses of 50 to 70 percent compared with areas that had little or no disease.

The fungus survives for many years in and on the soil as large masses of fungal tissue called sclerotia, which are about the size of wheat kernels. The sclerotia are formed by the fungal pathogen on and in soybean stems after stems are killed by the fungus. The pathogen does not impact the soybean plant until the soybean crop develops a sufficient canopy to shade the soil and lower portions of the plant and tiny mushroom-like spore-producing structures (apothecia) develop aerially from the sclerotia and produce spores that attack the plant via dead blossoms. The fungus is very aggressive and will rot the stem, killing the plant.

An increase in Sclerotinia stem rot has been associated with sustainable agriculture practices such as no-tillage or reduced tillage, and narrow crop row widths. Because conservation tillage practices leave crop residues on the surface and shade the stems and crop residue, the potential for an epidemic of white mold is increased. Reduced tillage concentrates the sclerotia in the top 2 to 4 inches of soil, where the sclerotia can germinate and produce apothecia. The dense canopy of narrow row soybeans offers a hospitable environment for development of the apothecia.

About 400 plant species are susceptible to infection by *S. sclerotiorum*, including soybeans, common beans, clovers, alfalfa, and most broadleaf weeds. Corn and small grains are rarely affected. Planting corn in alternate years will not help control *S. sclerotiorum* because the sclerotia can survive in the soil for many years.

Another fungus, *Sporidesmium sclerotivorum*, is an obligate parasite of the sclerotia of the *Sclerotinia* species. This mycoparasitic fungus has been developed by scientists at the USDA-ARS Biological Control of Plant Dis-



Symptoms of sclerotinia stem rot. Stem on right has sclerotia in stem.

eases Laboratory in Beltsville, Maryland, as an effective and economical biocontrol agent. The investigators' approach to control of the Sclerotinia stem rot problem in soybeans was to apply the mycoparasitic *S. sclerotivorum* to fields affected by the disease after the soybean crop was harvested. The fungus was allowed to kill the sclerotia of the soybean stem rot pathogen before a subsequent crop of soybeans was planted, and then continued to track the actual and cumulative effects over a two to three year period .

Objectives of this project were to:

- Evaluate the effectiveness of introducing *Sporidesmium sclerotivorum* into Iowa fields for the control of Sclerotinia stem rot of soybean,
- Determine the most economically and agronomically effective procedures for infestation of fields with inoculum of *Sporidesmium sclerotivorum* for control of Sclerotinia stem rot of soybean, and for inducing suppressiveness to *Sclerotinia sclerotiorum* in soil, and
- Isolate other parasites of the sclerotia of *Sclerotinia sclerotiorum* and evaluate them for effectiveness as biological control agents.

Approach and methods

The fungus *S. sclerotivorum* provided by the USDA Biological Control of Plant Diseases Laboratory was cultured for eight weeks and harvested. The inoculum was sprayed over the soil and plant debris with a backpack sprayer. After infestation, the top 2 inches of soil were mixed using a rototiller. Plots at each site were inoculated in the same manner.

Experiments were established in spring 1995 at three ISU research farms: the Hinds farm near Ames, the Kanawha farm in northern Iowa, and the Nashua farm in northeast Iowa. The fields at the Kanawha and Nashua farms were naturally infested with *S. sclerotiorum* and had been planted to soybeans in 1994. The experiments at all three sites had identical designs with different randomizations. Each plot was bordered on all sides by 10 feet of corn.

Each plot was infested with 600 ml of sclerotia of *S. sclerotiorum*. Additional inoculations were made at the Hinds and Kanawha sites. The weather at these sites was evidently too hot for good establishment of the pathogen, therefore, the researchers collected additional sclerotia from infested soybean seed and added them to the plots in the fall.

An additional plot was added near Humboldt in north central Iowa on a farm where Sclerotinia stem rot was very severe in 1995. After combining the soybean crop, the soybean debris was concentrated onto 10-ft by 10-ft squares with 10-ft borders.

The original plan for all three sites was to conduct research through the 1997 season, but poor weather for disease development prevailed in 1997 at all three sites, so an additional year of testing was added.

Soil samples were taken from all plots at all sites at the end of the 1996 and 1997 seasons. Fungi developing from the sclerotia were observed microscopically for signs of *S. sclerotivorum.*

Assessment of Sclerotinia sclerotiorum *activity* Plots planted to soybean were evaluated for signs of Sclerotinia stem rot at 10-day intervals in August and September. The number of plants and the number of plants showing symptoms of Sclerotinia stem rot were counted for three arbitrarily selected 1-meter row lengths and the percentage of diseased plants was calculated. Maximum disease incidence occurred about 90 days after planting.

When the plant canopy closed on the plots planted to soybeans, periodic arbitrary assessments of apothecial formation were begun.

The timing of the assessments was related to a great extent to the expected time of apothecial development following rainy periods. Counts were made of the number of sclerotia producing apothecia and number of apothecia per sclerotium, allowing researchers to estimate the pathogen's population and potential for disease development.

Demonstration plots Demonstration plots were located in soybean fields that experienced severe infestations of Sclerotinia stem rot. One was located on the Nashua research farm and the others were in commercial farmers' fields near Humboldt, Alexander, Nora Springs, and Conrad. The infested plots and surrounding areas were monitored for apothecial development after the canopy closed and for disease at 10-day intervals in August and early September.

Sampling of Iowa soils for mycopathogens of Sclerotia Germ-free sclerotia of *S. Sclerotiorum* were used as baits in the soil assays and for pathogenicity tests. Residual soil from 796 soil samples submitted to the ISU Plant Disease Clinic for Soybean Cyst Nematode assays was collected for this study. The samples came from 49 Iowa counties.
A pathogenicity test was conducted to confirm the parasitic nature of selected isolates of *Trichoderma* spp. and *Gliocladium* spp.

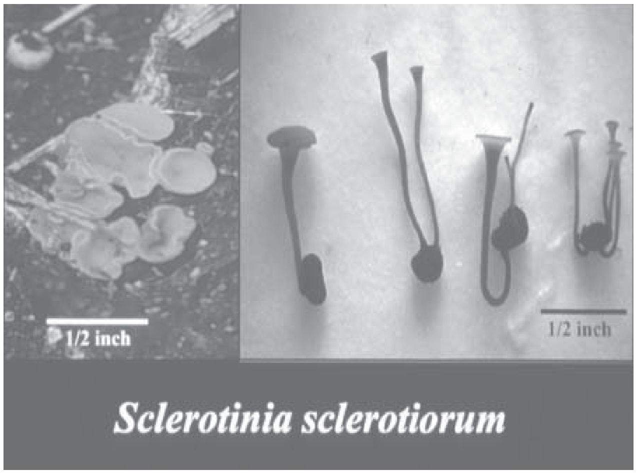
As a control, soils were also obtained from areas in Nebraska, Wisconsin, Minnesota, and North Dakota where it appears that *S. sclerotiorum* is no longer a problem. These soils were tested in a manner similar to the Iowa soils.

Results and discussion

Hinds farm experiment A July 1996 flash flood covered the Hinds farm plots with more than 2 feet of water. A few apothecia formed, but no disease developed. The field was chisel plowed in fall 1996 by mistake, so the experiment had to be reestablished with additional sclerotia added to all plots. In 1997 it was hot and dry and although sprinkler irrigation was used, no Sclerotinia stem rot was found. In 1998, cooler temperatures and irrigation resulted in a 17 percent infestation of Sclerotinia stem rot in the uninfested plots and a 65 percent decrease in Sclerotinia stem rot where *S. sclerotivorum* was infested at the higher rate. The amount of disease was related to the number of sclerotia germinating to produce apothecia. The flood of 1996 obviously spread the spores of the antagonist over the entire experiment and caused the originally uninfested plots to become infested with *S. sclerotivorum* in 1996.

Sporidesmium sclerotivorum *sporulating in the soil.*

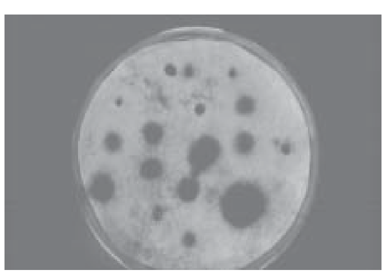
Humboldt farm experiment Incorporation of *S. sclerotivorum* spores into the plots in fall 1995 and spring 1996 resulted in an immediate decrease in the incidence of Sclerotinia stem rot in 1996. This was not associated with a decrease in the number of sclerotia *of S. Sclerotiorum* germinating into apothecia, but it was reflected in a decrease in the number of apothecia produced in plots infested at the high rate of *S. sclerotivorum* spores. A fall application of the antagonist resulted in a nearly 40 percent greater reduction of the germinating sclerotia and a parallel decrease in the number of apothecia compared to the spring application.



***Apothecia of* Sclerotinia sclerotivorum**

The hot, dry weather of 1997 did not allow for fruiting of the Sclerotinia stem rot fungus and no disease developed. All plots appeared to have about the same population of the antagonistic *S. sclerotivorum* at the end of the 1997 season. In 1998, hardly any Sclerotinia stem rot developed in the experimental area with an average of 0.2 percent of the plants showing Sclerotinia stem rot. Yet the area surrounding the experiment (about 15 to 20 yards away), planted to the same variety, had an average of 17 percent diseased plants.

Kanawha farm experiment The Sclerotinia stem rot at Kanawha appeared to be bit less where the high rates of *S. sclerotivorum* had been infested, compared to the uninfested control. Although fall applications of *S. sclerotivorum* seemed to decrease Sclerotinia stem rot by 40 percent over spring applications, the differences were not considered significant because of the high variability experienced at this site.



Sporidesmium sclerotivorum *growing out of sclerotia of the Sclerotinia stem rot fungus*

Hot, dry weather in 1997 prevented any apothecial development and Sclerotinia stem rot. The integrity of the plots was still intact at the end of the 1997 season as no *S. sclerotivorum* was found in soil samples from the uninfested control plots. Sclerotinia stem rot developed in the plots in 1998 with a 50 percent reduction in the level of disease in the *S. sclerotivorum* plots compared to the control plots.

Demonstration plots Three of the six demonstration plots areas observed for Sclerotinia stem rot in 1998 provided excellent examples of the potential of *S. sclerotivorum* for control of the disease. At the Alexander and Humboldt sites the disease was not observed in the infested areas and the influence of disease reduction was observed at least 5 meters (about 16 ft) beyond the borders of the infested areas. At the Conrad site infested in the fall, Sclerotinia stem rot incidence was suppressed greatly in the infested areas and again the influence of the disease suppression was observed at least 5 meters beyond the edge of the infestation.

The Nora Springs site was compromised when the cooperating farmer rented the field, including our research area, to a commercial seed company to test its varieties for resistance to Sclerotinia stem rot. Very little Sclerotinia stem rot developed in the field at Nashua that was infested with *S. sclerotivorum*, so we could not measure any effects on the farm. The demonstration plots on the second Conrad farm, which were infested in spring 1997, did not show any significant benefit from the infestation.

The demonstration plots provided data that are probably better examples of the potential of *S. sclerotivorum* as a biocontrol agent than the experiments at Hinds and Kanawha. The Humboldt experiment essentially turned into a demonstration plot when all the other small plots became contaminated with *S. sclerotivorum.* An encouraging fact is that the antagonistic *S. sclerotivorum* remained at an apparently high population level in the infested soils. The marginal results at the second Conrad site and the Nora Springs site provide evidence that factors limiting *S. sclerotivorum* growth and development may be affecting its disease control benefits.

Mycopathogens of sclerotia in Iowa soils Trichoderma spp. were the most frequently isolated mycoparasites with 119 isolates retrieved from 34 of the 49 counties surveyed, however it is not an aggressive pathogen of sclerotia like *S. sclerotivorum.* Nine samples of *S. sclerotivorum* were found in eight of the 49 counties. Whenever present, *S. sclerotivorum* parasitized more than 50 percent of the sclerotia baited to the soil sample. If pure strains can be obtained from these samples, this local source of *S. sclerotivorum* may be better adapted to use in Iowa than the strain used in the studies.

Conclusions

The results of this study established that *Sporidesmium sclerotivorum* was able to effectively control Sclerotinia stem rot of soybeans in Iowa. *Sporidesmium sclerotivorum* is a mycopathogen of the sclerotia of *Sclerotinia sclerotiorum,* which are the survival structures of this causal agent of Sclerotinia stem rot. The spores of *Sporidesmium sclerotivorum* were applied to soil and soybean debris after a harvested crop of soybeans was removed. When soybeans were replanted to the field two to three years later, the *Sporidesmium sclerotivorum* fungus had killed a sufficient population of sclerotia to reduce the disease potential to the point where very little Sclerotinia stem rot developed. The effectiveness was reflected in the decreased fruiting of the Sclerotinia stem rot fungus.

Where the *Sporidesmium sclerotivorum* apparently was less effective in controlling stem rot, it appears excessive tillage had brought the sclerotia of Sclerotinia not previously exposed to *Sporidesmium sclerotivorum* to the soil surface. Producers will need to identify appropriate tillage practices to be used after infesting the soil with *Sporidesmium sclerotivorum* to maximize chances for exposure of sclerotia to the spores of *Sporidesmium sclerotivorum.*

The optimum temperatures for *Sporidesmium sclerotivorum* spore germination were 68 to 86 degrees F, and the optimum soil pH values were 4.5 to 6.5. However, most of the experiments in this study were conducted in soils of greater than 6.5 pH and *Sporidesmium sclerotivorum* still was a very effective deterrent to the stem rot fungus. Further study will help determine at which point pH may be a limiting factor in successful biocontrol.

The fungus *Sporidesmium sclerotivorum* has great potential for use in controlling Sclerotinia stem rot in Iowa soybeans and the next step is developing a system for commercial production of the inoculum and obtaining EPA and USDA approval for applying the mycoparasite to Iowa soils. A survey of Iowa soils showed that *Sporidesmium sclerotivorum* was a natural component of nine of the 796 Iowa soil samples, so adding the *Sporidesmium sclerotivorum* spores would be a matter of augmenting a native organism which will make it easier to obtain permission for commercial use.

Impact of results

Sporidesmium sclerotivorum is an environmentally friendly organism that has proved effective in controlling Sclerotinia stem rot in Iowa soybeans. The use of this biocontrol agent will allow for continued use of soil conservation practices and narrow row widths in soybean production. It has a high potential to displace fungicides and herbicides used to control the disease and should be residual from year to year, thereby rendering soils suppressive to the stem rot fungus. Use of *Sporidesmium sclerotivorum* to deter fungus will make it less important to attempt to breed soybean seed with resistance to the stem rot fungus.

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Education and outreach

Thus far, these research findings have been disseminated primarily to scholarly groups: the American Phytopathological Society, the Entomological Society of America, and the Canadian Phytopathological Society. Reports have been made at USDA Regional meetings and at a National Sclerotinia Workshop held in Michigan. A poster presentation at the 1998 ISU Agronomy Days was well-received with much interest in the *Sporidesmium sclerotivorum* inoculum.